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by life technologies™

pShooter™ Vector (pEF/myc vector)

For Intracellular Targeting of Recombinant Proteins and Antibodies

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User Manual

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Kit Contents and Storage

Contents

The pShooter™ manual for vectors utilizing the EF-1α promoter and the *c-myc* epitope is included with the following vector.

Vector	Amount	Catalog no.
pEF/*myc*/nuc pEF/*myc*/nuc/GFP	20 µg each (40 µl of 0.5 µg/µl vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	V891-20

Shipping/Storage

All vectors are shipped at room temperature. Upon receipt, store at –20°C.

Products Available Separately

Primers to sequence your insert in the pEF/*myc* vectors and antibodies to the *c-myc* epitope are available from Invitrogen. In addition, Geneticin® is also available for selection of stable cell lines. Lastly, pShooter™ vectors containing the CMV promoter are also available. You may find that one promoter expresses your protein better than the other in your particular cell line. See the table below for ordering information.

Vector	Amount	Catalog no.
pEF Forward Primer	2 µg, lyophilized	N623-02
BGH Reverse Primer	2 µg, lyophilized	N575-02
Anti-*myc* Antibody	25 Westerns	R950-25
Anti-*myc*-HRP Antibody	25 Westerns	R951-25
Geneticin®	1 g	11811-023
	5 g	11811-031

Introduction

Product Description

Background

The final location of a protein within a cell depends upon 'targeting sequences' encoded within the sequence of a protein. The presence of a nuclear localization sequence within a protein or at the N- or C-terminus, directs the protein to the nucleus.

Description

The pShooter™ vector is designed to express and target your recombinant protein to the desired intracellular location in mammalian cells. They were originally designed to target single-chain antibodies (scFvs) to specific intracellular locations (Persic *et al.*, 1997a; Persic *et al.*, 1997b). The pShooter™ vector described in this manual is a 5.5 kb expression vector that expresses your recombinant protein as a fusion to a targeting sequence (if necessary). Proteins are targeted to the nucleus (Fisher-Fantuzzi & Vesco, 1988). Expression is driven by the strong, constitutive human EF-1α promoter (Mizushima & Nagata, 1990). The table below summarizes the above features.

Vector	Desired Location	Targeting Signal(s)
pEF/*myc*/nuc	Nucleus	3X (DPKKKRKV)

In addition, the vector uses the same backbone (pcDNA3) which includes the bovine growth hormone polyadenylation sequence, an f1 origin, the SV40 origin, the neomycin resistance gene, the SV40 late polyadenylation sequence, pUC origin, and the ampicillin resistance gene (Persic *et al.*, 1997b). For more information on all of the above features, see page 15 in the Appendix.

Uses of the pShooter™ Vector

Targeting Recombinant Proteins

The vector can be used to direct any recombinant protein to a particular intracellular location. However, success may be dependent on the specific protein used. To help analyze experiments, the vector is supplied with an optimized form of green fluorescent protein (cycle 3 GFP) cloned into the vector as a control. Guidelines for assaying cycle 3 GFP fluorescence are also provided (see page 12).

Continued on next page

Product Description, continued

Uses of the pShooter™ Vector, continued

Targeting Antibodies

The pShooter™ vector was originally designed for the targeting of scFvs to a specific intracellular location for intracellular immunization (Biocca & Cattaneo, 1995; Cattaneo & Biocca, 1997; Persic *et al.*, 1997a). In this technique, an antibody which is inhibitory for a protein's function can be directed to the same compartment as the protein itself to inactivate the protein. The pShooter™ vector retains all of the features cited in Persic, *et al.*, 1997a. Some of these features are summarized below.

- The restriction sites in the multiple cloning site were chosen because they are rare in both human and mouse antibody variable regions and have been removed from the rest of the vector.
- Vectors consist of a number of functional cassettes flanked by unique restriction sites, with junctional DNA reduced to a minimum.
- The nuclear localization signal is designed to be at the C-terminus of a scFv, positioned away from the antigen binding site, to reduce potential problems of steric hindrance.
- scFvs derived from phage antibody libraries can be easily cloned in from compatible vectors (e.g. pHEN; (Hoogenboom *et al.*, 1991)) or amplified incorporating compatible ends.

For more information on cloning antibodies and antibody domains, refer to Persic, *et al.*, 1997a. For an example in which these vectors have been used in intracellular immunization to inhibit function within a cell, see Gargano and Cattaneo, 1997.

Methods

General Guidelines

Introduction

This section contains general information on propagation and maintenance of the pShooter™ vector and guidelines for *E. coli* transformation. Additional information is provided on the following pages:

- To develop a cloning strategy, refer to the multiple cloning sites on page 5.
- A map of the targeting vector is on page 14.
- A map of the control vector is on page 15.
- Full sequences of any of the vectors described in this manual may be obtained by downloading them from our Web site (www.invitrogen.com) or by calling Technical Support (see page 17).

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, purification of single-stranded DNA, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994) (See **References**, page 19).

E. coli Strain

Many *E. coli* strains are suitable for the growth of this vector including TOP10F´ (Catalog no. C615-00) and DH10B™. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10F´ and DH10B™ are available as chemically competent or electrocompetent cells (TOP10F´ only) from Invitrogen.

Item	Quantity	Catalog no.
One Shot™ TOP10F´ (chemically competent cells)	21 x 50 µl	C3030-03
Max Efficiency® DH10B™ (chemically competent cells)	5 x 0.2 ml	18297-010
Electrocomp™ TOP10F´	5 x 80 µl	C665-55

E. coli Transformation

You may use any method you wish to prepare competent *E. coli* for transformation. Select transformants on LB plates containing 50-100 µg/ml ampicillin.

Continued on next page

General Guidelines, continued

Propagation and Maintenance of Plasmids

In order to propagate and maintain the pShooter™ vector, we recommend that you transform the plasmids into *E. coli* and prepare glycerol stocks for long-term storage. Transform plasmids into *E. coli* as follows:

- Use a small amount of the supplied vector stock solution and the transformation method of choice to transform a *rec*A, *end*A *E. coli* strain like TOP10F´, DH10B™, INVαF´, DH5αF´, or equivalent.
- Select transformants on LB plates containing 50-100 µg/ml ampicillin.
- Select a transformant and grow a log phase culture for a glycerol stock.
- Prepare glycerol stocks by mixing 0.85 ml of the log phase culture with 0.15 ml of sterile glycerol.
- Transfer the resulting solution to a cryovial and store at -80°C.

Cloning into the pShooter™ Vectors

Diagram for the multiple cloning sites is provided on page 5 to help you clone your gene of interest in frame with the desired targeting signal and/or the *c-myc* epitope for detection. For help with PCR, restriction digests, and ligations, refer to general molecular biology texts (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989).

Transform ligation mixtures into competent *E. coli* as using the method of choice, and plate the cells on LB plates containing 50-100 µg/ml ampicillin.

Select 10 to 20 transformants and analyze your construct by restriction enzyme digestion or sequencing to ensure that your insert is cloned in the correct orientation. If you wish to sequence your insert, use the pEF Forward and BGH Reverse primers (Catalog nos. N623-02 and N575-02, respectively) to confirm that your gene is correctly fused to the targeting signal and/or the *c-myc* epitope.

Cloning into pEF/*myc*/nuc

Special Considerations

The ATG in the *Nco* I site is part of a Kozak consensus sequence (ANNATGG) (Kozak, 1987; Kozak, 1990). If you can clone in frame or flush with this ATG, it will facilitate expression of your protein.

To efficiently target your protein to the nucleus, the nuclear localization signal (NLS) from SV40 large T antigen has been triplicated and placed downstream of the multiple cloning site for C-terminal fusion to your protein (Fisher-Fantuzzi & Vesco, 1988). Note that this signal will not be removed from your protein upon entry to the nucleus. If you clone in-frame with the NLS you will also be in frame with the *c-myc* epitope. The NLS and the *c-myc* epitope will add ~5 kDa to your protein.

Note that you may have to use PCR to facilitate in-frame cloning with the ATG (if desired) and the NLS.

pEF/*myc*/nuc MCS

The multiple cloning site below shows part of the 5′ untranslated region from the EF-1α promoter. For more information on the EF-1α promoter, see page 13. Restriction sites are labeled to indicate the cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing.

pEF Forward

```
1081  ATGTAATTCT CCTTGGAATT TGGCCTTTTT GAGTTTGGAT CTTGGTTCAT TCTCAAGCCT
```

priming site; 3′ end of EF-1α Intron 1; *Nco* I

```
1141  CAGACAGTGG TTCAAAGTTT TTTTCTTCCA TTTCAGGTGT CGTGAACACG TGGCCACC ATG GCC
                                        5′ End of EF-1α Exon 2          Met Ala
```

Pst I *Sal* I; *Xho* I; *Not* I; NLS #1

```
1205  CAG GTG CAG CTG CAG GTC GAC CTC GAG ATC AAA CGG GCG GCC GCA GAT CCA AAA
      Gln Val Gln Leu Gln Val Asp Leu Glu Ile Lys Arg Ala Ala Ala Asp Pro Lys
```

NLS #2; NLS #3

```
1259  AAG AAG AGA AAG GTA GAT CCA AAA AAG AAG AGA AAG GTA GAT CCA AAA AAG AAG
      Lys Lys Arg Lys Val Asp Pro Lys Lys Lys Arg Lys Val Asp Pro Lys Lys Lys
```

myc epitope

```
1289  AGA AAG GTA GAT ACG GCC GCA GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT
      Arg Lys Val Asp Thr Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
```

BGH Reverse priming site

```
1367  GGG GCC GCA TAG TCTAGAAGCT CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT
      Gly Ala Ala ***
```

```
1427  CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT TGACCCTGGA AGGTGCCACT CCCACTGTCC
```

BGH polyadenylation signal

```
1487  TTTCCTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG
```

Transfection of Mammalian Cells

Introduction

General information is provided below for transfection of mammalian cells with the pShooter™ vector. Positive control vector is supplied with each vector to optimize transfection conditions for your cell line. pShooter™ vectors have been tested in Chinese Hamster Ovary (CHO) and COS cells. A sample transfection is provided on the next page for CHO cells.

Plasmid Preparation

Once you have confirmed that your gene is in the correct reading frame, prepare plasmid DNA for transfection. Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells and salt will interfere with lipid complexing decreasing transfection efficiency. We recommend isolating DNA using the S.N.A.P.™ MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01) or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (e.g. COS, CHO), consult original references or the supplier of your cell line for the optimal method of transfection. It is recommended that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Methods for transfection include calcium phosphate (Chen & Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner & Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa & Dower, 1988). Invitrogen offers the Calcium Phosphate Transfection Kit for mammalian transfection and Lipofectamine™ 2000 Reagent for lipid-mediated transfection.

Catalog No.	Description	Quantity
11668-019	Lipofectamine™ 2000 Reagent	1.5 ml

Expression of Your Fusion Protein

No matter which method of transfection you elect to use, it is very important to perform a time course to optimize expression and targeting of your particular protein. Be sure to transfect enough cells to collect time points, particularly if you are using immunofluorescence or a functional assay.

Methods of Detection

There are a variety of methods for detection, depending on what protein you are expressing and targeting.

- **Visual Method**. If you want to be sure that your protein is targeting to the correct location, use immunofluorescence (see page 9).
- **Functional Assay**. If you are targeting a protein that inhibits or alters the function of another protein, you may have a visual assay (e.g. changes in cell morphology) or an enzymatic assay.

Continued on next page

Transfection of Mammalian Cells, continued

Sample Transfection Procedure

Below is a sample transfection procedure using Lipofectamine™ 2000, available from Invitrogen (Catalog no. 11668-019) and a GFP control vector.

Day 1

1. Plate 2 x 10^5 CHO KI cells in 3 ml F12 Nutrient mixture, Ham, 10% FBS into one 60 mm plate.

Day 2

2. Prepare plasmid DNA lipid complexes according to the instructions provided in the Lipofectamine™ 2000 Reagent manual, which is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (see page 17).
3. Add the DNA lipid complexes to the cells.
4. Incubate the cells at 37°C in a CO_2 incubator.

Days 3, 4, and 5

Monitor fluorescence of GFP with a fluorescent microscope as described on page 12, and take photographs at 24, 48, and 72 hours.

Stable Transfection (Geneticin® Resistance)

For stable transfection, the pShooter™ vector contains the resistance factor to Geneticin®. Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression in mammalian cells of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, results in detoxification of Geneticin® Selective Antibiotic (Southern & Berg, 1982).

Geneticin® Selection Guidelines

Geneticin® is available from Invitrogen (Catalog no. 11811-031). Use as follows:

1. Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
2. Use 100 to 1000 µg/ml of Geneticin® in complete medium.
3. Calculate concentration based on the amount of active drug (check the lot label).
4. Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin®.

Cells will divide once or twice in the presence of lethal doses of Geneticin® Selective Antibiotic, so the effects of the drug take several days to become apparent. Complete selection can take from 2 to 4 weeks of growth in selective medium.

Continued on next page

Transfection of Mammalian Cells, continued

Linearization of Vectors for Stable Transfection

While linearizing a plasmid is not necessary to obtain stable transfectants, it ensures that the vector does not integrate in a way that disrupts the gene of interest. Possible restriction enzymes you could use to linearize your particular construct are listed below.

Vector	Sites	Location
All vectors	*Pvu* I, *Sca* I	Ampicillin resistance gene
pEF/*myc* vectors	*Kpn* I, *Eco*R I, *Hin*d III	5´ end of EF-1α promoter
pEF/*myc* GFP control vectors	*Kpn* I, *Eco*R I	5´ end of EF-1α promoter

Detection of Fusion Proteins

Introduction

To ensure that your protein is targeted correctly, it is important to visualize its cellular location. Inclusion of the *c-myc* epitope allows detection by immunofluorescence although you can use antibody to your own protein. A basic protocol is included for your convenience. Other protocols may be appropriate.

Detection of Fusion Proteins

Antibodies to the *c-myc* epitope are available from Invitrogen and can be used to detect expression of your fusion protein by immunofluorescence (see below) or Western blot. **Note that the *c-myc* epitope will add an additional 1.5 kDa to your protein.** The table below describes the antibodies available and ordering information. The amount supplied is sufficient for 25 Westerns and 2-3 immunofluorescence experiments.

Antibody	Purpose	Catalog no.
Anti-*myc*	Detects 10 amino acid epitope derived from the *c-myc* protein (Evan *et al.*, 1985)	R950-25
Anti-*myc*-HRP	See above. Provided as an HRP conjugate for time-saving detection.	R951-25

Basic Immunofluorescent Labeling of Cells

Antibodies can be used for immunofluorescence using standard techniques (Ausubel *et al.*, 1994). A basic protocol is supplied below for adherent cells. For more information, refer to Chapter 14.6 in *Current Protocols in Molecular Biology*.

1. Cool the cells on ice. (Culture cells in a 3- to 5-cm dish. Cells should be confluent or as close to confluent as possible.)
2. Aspirate off the culture medium and wash the cells with +4°C PBS.
3. Remove PBS and fix cells for either 30 minutes in 2% paraformaldehyde/0.1% Triton X-100 or 15 minutes in 100% methanol at -20°C. **Note**: Be sure to wash the cells thoroughly with methanol or they will freeze.
4. Remove fixative and wash the cells twice with cold PBS (~5 minutes/wash).
5. Dilute primary antibody in PBS to a final concentration of 5 to 10 µg/ml. Prepare enough antibody to cover cells.
6. Centrifuge antibody for 2 minutes at 13,500 x g (+4°C) to precipitate any particulate matter.
7. Carefully layer primary antibody onto the cells until they are just covered and incubate for 1 hour at +4°C.
8. Remove antibody and wash four times with cold PBS (~5 minutes/wash).
9. Dilute labeled secondary antibody in PBS to a final concentration of 5 to 10 µg/ml. Prepare enough antibody to cover cells.
10. Centrifuge antibody for 2 minutes at 13,500 x g (+4°C) to precipitate any particulate matter.
11. Layer secondary antibody over cells and incubate for 1 hour at +4°C.
12. Remove antibody and wash four times with cold PBS (~5 minutes/wash). Store cells in PBS. Analyze cells by fluorescence immediately; or cover dishes, wrap in aluminum foil, and refrigerate. Be sure to examine preparations within 24 hours or the fluorescence will fade.

Continued on next page

Detection of Fusion Proteins, continued

Patterns of Expression

Transformation of the vector expressing your gene of interest or the control vectors should give the following expression patterns using immunofluorescence or fluorescence (cycle 3 GFP).

Nuclear expression: Recombinant proteins should be primarily localized to the nucleus.

If you have trouble expressing and targeting your protein, read the section on the positive control vectors below and the **Troubleshooting** section on page 11.

Note

Color graphics are available in our Catalog showing expression and targeting of cycle 3 GFP in CHO cells. In addition, the original paper by Persic, et al., shows targeting of recombinant antibodies by immunofluorescence using the monoclonal antibody 9E10 (Anti-*myc* Antibody) in COS cells (Persic *et al.*, 1997a).

Using the Positive Controls

Each of the pShooter™ vectors described in this manual is also provided with a control vector expressing cycle 3 GFP. These vectors may be used to:

- Optimize transfection conditions for your cell line
- Confirm that the targeting signals function properly in your cell line

For more information on the control vector, see page 15. For information on detection of cycle 3 GFP, see page 12.

Troubleshooting

Troubleshooting

Use the table below to troubleshoot expression and targeting.

Problem	Reason	Solution
No targeting observed	Low expression levels	Could be a variety of reasons. Check for expression by Western blot. You may have to optimize transfection conditions (use the cycle 3 GFP control vector to evaluate transfection). Many of the other solutions below may help.
	No expression of your protein	Check for expression by Western blot. If your protein is not expressed, sequence your construct to confirm that it is in frame with the targeting sequence.
	Cell line may not recognize targeting signal	Check for targeting using the appropriate GFP control vector.
Non-specific labeling	The *c-myc* tag is derived from an endogenous protein (*c-myc*)	Transfect with the empty vector (negative control) and assay for immunofluorescence. You may need to use a different tag or use antibody to your protein
Difficulty expressing protein in stable clones	Protein is toxic when redirected to another compartment	Selection of stable clones may lead to down regulation of the protein. Try a different promoter for expression.
	Continuous culture may lead to loss of protein expression	Remember to prepare an early set of back-up stocks.

Note

Some proteins (e.g., antibodies expressed intracellularly) may give a very good immunofluorescent signal, but may not be detectable in a Western blot. This may be due to aggregation and/or precipitation of the antibody, so be sure your SDS-PAGE samples are well solubilized.

Detection of GFP

Introduction

Cycle 3 GFP has been optimized for expression in *E. coli* and mammalian cells. Fluorescent yield is >40-fold over wild-type GFP, yet it has the same excitation maxima (395 nm and 478 nm for primary and secondary excitation) and emission maxima (507 nm). Guidelines for detection and optimization of expression are described below.

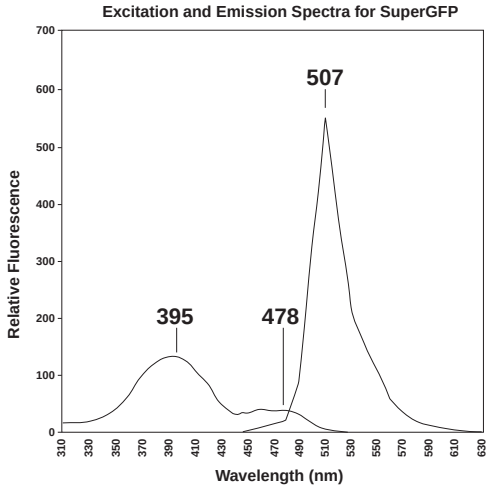
Construct of the Control Vectors

The control vectors were synthesized by amplifying a 716 bp fragment from pαGFP (Crameri *et al.*, 1996) using oligomers that introduced a *Pst* I site at the 5´ end and a *Not* I site at the 3´ end of cycle 3 GFP. In addition each of the oligomers was specifically designed to clone in frame with the targeting sequence and/or the *c-myc* epitope.

Detection of Fluorescence

To detect fluorescent cells, it is important to pick the best filter set to optimize detection. The primary excitation peak of cycle 3 GFP is at 395 nm. There is a secondary excitation peak at 478 nm. Excitation at these wavelengths yields a fluorescent emission peak with a maximum at 507 nm (see below).

Use of the best filter set will ensure that the optimal regions of the cycle 3 GFP spectra are excited and passed (emitted). For example, the FITC filter set that we use excites cycle 3 GFP with light from 460 to 490 nm, which covers the secondary excitation peak. The filter set passes light from 515 to 550, allowing detection of most of the GFP fluorescence. Standard FITC filters easily suit most purposes; however, it is important to keep in mind that fluorescence will be affected by the sample assayed and the filter you choose



For general information about GFP fluorescence and detection, refer to Current Protocols in Molecular Biology.

Detection of Transfected Cells

After transfection, allow the cells to recover for 12 to 48 hours before assaying for fluorescence. **Note**: Most media fluoresce because of the presence of riboflavin (Zylka & Schnapp, 1996) and may interfere with detection of cycle 3 GFP fluorescence. Medium can be removed and replaced with PBS to alleviate this problem.

Estimate the total number of cells before assaying for fluorescence. Then check your plate for fluorescent cells. You can use fluorescence to estimate transfection efficiency and normalize any subsequent assay for your gene of interest.

Optimizing Expression

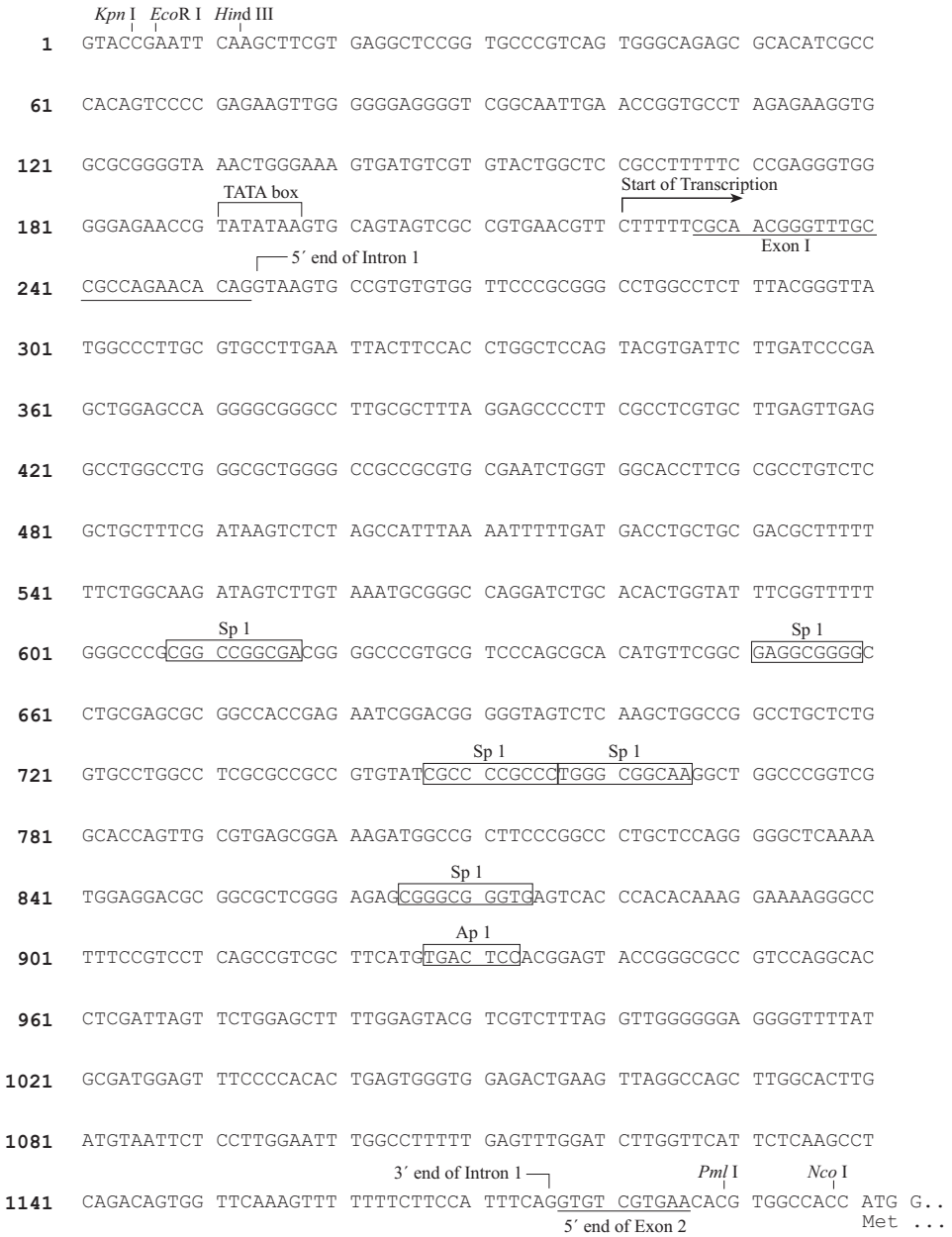
It is recommended that a time course be performed to determine the optimal time to assay for transient expression of GFP. **Optimal times may vary from 12 to 96 hours from the time of transfection depending on cell line.**

Appendix

EF-1α Promoter

Description

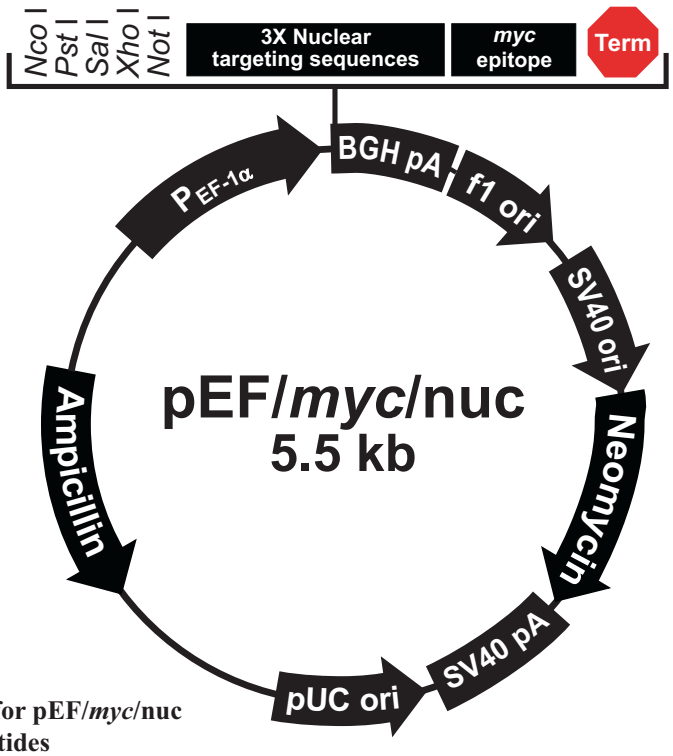
The diagram below shows all the features of the EF-1α promoter used in the pShooter™ vector (Mizushima & Nagata, 1990). Features are marked as per Uetsuki, *et al.*, 1989. The original sequence has been mutagenized to remove the *Pst* I, *Bgl* II, *Afl* II, and *Xho* I restriction sites. In addition, *Kpn* I, *Eco*R I, *Hin*d III, *Pml* I, and *Nco* I were introduced by PCR. The EF-1α promoter can be excised using *Kpn* I, *Eco*R I, or *Hin*d III and *Pml* I or *Nco* I.


Kpn I
EcoR I
Hind III
1 GTACCGAATT CAAGCTTCGT GAGGCTCCGG TGCCCGTCAG TGGGCAGAGC GCACATCGCC
61 CACAGTCCCC GAGAAGTTGG GGGGAGGGGT CGGCAATTGA ACCGGTGCCT AGAGAAGGTG
121 GCGCGGGGTA AACTGGGAAA GTGATGTCGT GTACTGGCTC CGCCTTTTTC CCGAGGGTGG
TATA box
Start of Transcription
181 GGGAGAACCG TATATAAGTG CAGTAGTCGC CGTGAACGTT CTTTTTCGCA ACGGGTTTGC
Exon I
5′ end of Intron 1
241 CGCCAGAACA CAGGTAAGTG CCGTGTGTGG TTCCCGCGGG CCTGGCCTCT TTACGGGTTA
301 TGGCCCTTGC GTGCCTTGAA TTACTTCCAC CTGGCTCCAG TACGTGATTC TTGATCCCGA
361 GCTGGAGCCA GGGGCGGGCC TTGCGCTTTA GGAGCCCCTT CGCCTCGTGC TTGAGTTGAG
421 GCCTGGCCTG GGCGCTGGGG CCGCCGCGTG CGAATCTGGT GGCACCTTCG CGCCTGTCTC
481 GCTGCTTTCG ATAAGTCTCT AGCCATTTAA AATTTTTGAT GACCTGCTGC GACGCTTTTT
541 TTCTGGCAAG ATAGTCTTGT AAATGCGGGC CAGGATCTGC ACACTGGTAT TTCGGTTTTT
Sp 1
Sp 1
601 GGGCCCGCGG CCGGCGACGG GGCCCGTGCG TCCCAGCGCA CATGTTCGGC GAGGCGGGGC
661 CTGCGAGCGC GGCCACCGAG AATCGGACGG GGGTAGTCTC AAGCTGGCCG GCCTGCTCTG
Sp 1
Sp 1
721 GTGCCTGGCC TCGCGCCGCC GTGTATCGCC CCGCCCTGGG CGGCAAGGCT GGCCCGGTCG
781 GCACCAGTTG CGTGAGCGGA AAGATGGCCG CTTCCCGGCC CTGCTCCAGG GGGCTCAAAA
Sp 1
841 TGGAGGACGC GGCGCTCGGG AGAGCGGGCG GGTGAGTCAC CCACACAAAG GAAAAGGGCC
Ap 1
901 TTTCCGTCCT CAGCCGTCGC TTCATGTGAC TCCACGGAGT ACCGGGCGCC GTCCAGGCAC
961 CTCGATTAGT TCTGGAGCTT TTGGAGTACG TCGTCTTTAG GTTGGGGGGA GGGGTTTTAT
1021 GCGATGGAGT TTCCCCACAC TGAGTGGGTG GAGACTGAAG TTAGGCCAGC TTGGCACTTG
1081 ATGTAATTCT CCTTGGAATT TGGCCTTTTT GAGTTTGGAT CTTGGTTCAT TCTCAAGCCT
3′ end of Intron 1
Pml I
Nco I
1141 CAGACAGTGG TTCAAAGTTT TTTTCTTCCA TTTCAGGTGT CGTGAACACG TGGCCACC ATG G..
5′ end of Exon 2
Met ...

pEF/*myc*/nuc Map

Map

The figure below summarizes the features of pEF/*myc*/nuc. The complete nucleotide sequence for pEF/*myc*/nuc is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (see page 17).



Comments for pEF/*myc*/nuc
5536 nucleotides

EF-1α promoter: bases 6-1192
EF Forward priming site: bases 1133-1153
Multiple cloning site: bases 1197-1248
Nuclear localization site (3X): bases 1250-1273, 1274-1297, 1298-1321
myc epitope: bases 1334-1363
BGH Reverse priming site: 1399-1416
BGH polyadenylation sequence: bases 1398-1612
f1 origin: bases 1675-2088
SV40 promoter/origin: bases 2153-2460
Neomycin (G418) resistance gene (ORF): bases 2468-3262
SV40 polyadenylation sequence: bases 3279-3517
pUC origin: bases 3700-4373 (Complementary strand)
Ampicillin resistance gene (ORF): 4518-5378 (Complementary strand)

Features of pEF/*myc*/nuc

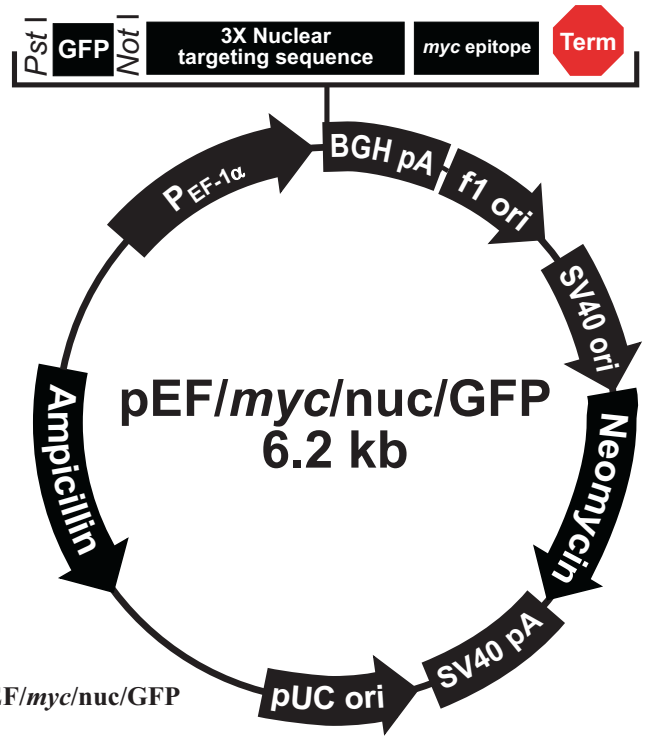
Table The table below summarizes the features of the pShooter™ vector, derived from pcDNA3. Changes to the vector backbone are noted.

Feature	Benefit
Human EF-1α promoter	Permits efficient, high-level expression of your recombinant protein (Kim *et al.*, 1990; Mizushima & Nagata, 1990; Uetsuki *et al.*, 1989). For more detailed information on this promoter, see the next page.
Multiple cloning site	Allows insertion of your gene.
Nuclear targeting sequence pEF/*myc*/nuc only	Permits efficient targeting of your protein to the nucleus. Sequence is triplicated to ensure proper localization. Isolated from SV40 large T antigen (Fisher-Fantuzzi & Vesco, 1988).
c-myc epitope (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of your recombinant protein by immunofluorescence with the Anti-*myc* Antibody (Catalog no. R950-25) (Evan *et al.*, 1985).
ER retention signal pEF/*myc*/ER only	Permits retention of your protein in the ER (Munro & Pelham, 1987).
TAG termination codon	For efficient termination of translation.
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin & Rottman, 1992).
f1 origin	Allows rescue of single-stranded DNA.
SV40 early promoter and origin	Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen (i.e. COS). *Nco* I site removed by site-directed mutagenesis.
Neomycin resistance gene	Selection of stable transfectants in mammalian cells (Southern & Berg, 1982). Tn*5* sequence removed and the Kozak sequence improved by PCR at the 5´ end of the ORF. *Nco* I, *Pst* I, and *Bss*H II sites removed by site-directed mutagenesis.
SV40 polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA.
pUC origin	High-copy number replication and growth in *E. coli*. *Apa*L I site removed by site-directed mutagenesis.
Ampicillin resistance gene (β-lactamase)	Selection of vector in *E. coli*. *Apa*L I site removed by site-directed mutagenesis.

pEF/*myc*/nuc/GFP Map

Map

The figure below summarizes the features of pEF/*myc*/nuc/GFP. The complete nucleotide sequence for pEF/*myc*/nuc/GFP is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Support (page 17).



Comments for pEF/*myc*/nuc/GFP
6232 nucleotides

EF-1α promoter: bases 6-1192
EF Forward priming site: bases 1133-1153
GFP ORF: bases 1220-1936
Nuclear localization site (3X): bases 1946-1969, 1970-1993, 1994-2017
myc epitope: bases 2030-2059
BGH Reverse priming site: 2095-2112
BGH polyadenylation sequence: bases 2094-2308
f1 origin: bases 2371-2784
SV40 promoter/origin: bases 2849-3156
Neomycin (G418) resistance gene (ORF): bases 3164-3958
SV40 polyadenylation sequence: bases 3975-4213
pUC origin: bases 4396-5069 (Complementary strand)
Ampicillin resistance gene (ORF): 5214-6074 (Complementary strand)

Technical Support

Web Resources

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
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